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SYNTHESIS OF NEW PYRIDINE DERIVATIVES AS POTENT ANTIFUNGAL AGENTS.

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ABSTRACT

A series of 2-aminomethylene-[2´-(3´´-chloro-2´´-oxo-4´´-substitutedaryl-1´´-azetidinyl)-1´,3´,4´-thiadiazol-5´-yl)pyridines (5a-5g) and 2-aminomethylene-[2´-(2´´-substitutedaryl-4´´-thiazolidinone-3´´-yl)-1´,3´,4´-thiadiazol-5´-yl]pyridines (6a-6g) has been synthesised from 2-aminomethylene-(2´-substitutedarylidineimino-1´,3´,4´-thiadiazol-5´-yl)pyridines (4a-4g). The structures of all these compounds were established on the basis of elemental (C,H,N) and spectral (IR, ^{1}H-NMR and mass spectral data) analysis. All the compounds were screened for their antifungal activity. Compound 2-aminomethyene-[2´-(2´´-(3-hydroxyphenyl)-4´´-thiazolidinone-3´´-yl)-1´,3´,4´-thiadiazol-5´-yl]pyridines were found to be the most potent compound of the series and its activity was compared with the reference drugs Fluconazole and Gieseofulvin.

KEY WORDS

Pyridine, thiadiazole, thiazolidinone, azetidinone and antifungal activity.

INTRODUCTION

Pyridine derivatives of different heterocyclic nucleus have shown potent pharmacological properties like antifungal[1-3], antitubercular[4], antibacterial[5], antimicrobial[6], insecticidal[7] etc. Furthermore, different congeners of thiadiazole[8-9], thiazolidinone[10-11], and azetidinone[12-13] have also been reported to exhibit potent antifungal activities by several scientists. In the light of these observations, compounds of series I were synthesized incorporating thiadiazole, thiazolidinone, and azetidinone moieties at 2-position of pyridine nucleus with a hope to develop better antifungal agents. These compounds have been screened for their antifungal activity.

Chemistry

The synthetic routes of compounds are outlined in Scheme I. As shown in Scheme I compound 1 i.e 2 ethylaminopyridinoacetate was formed by the reaction of starting material i.e 2-aminopyridine with ethylchloroacetate and anhydrous K_2CO_3, which further reacted with thiosemicarbazide to give 2-aminopyridineacetylthiosemicarbaide (compound 2). Compound 2 when treated with conc. H_2SO_4 and then neutralized with liquid ammonia resulted in the formation of compound 3 i.e 2-aminomethylene-(2´-amino-1´,3´,4´-thiadiazol-5´-yl)pyridine, which when treated with different substituted aromatic aldehydes formed various substituted arylidine derivatives (4a-4d). These arylidine derivatives on treatment with triethylamine and chloroacetylchloride yielded different azetidinyl derivatives i.e compound 5a-5d. On the other hand, these arylidine derivatives on reaction with thioglycolic acid and anhydrous $ZnCl_2$ furnished various thiazolidinone derivatives i.e compound 6a-6d.

RESULT AND DISCUSSION

Twenty two compounds (**3, 4a-4g, 5a-5g**, and **6a-6g**) and the standard drugs i.e fluconazole and griseofulvin were screened for their antifungal activity at a concentraton of 100 mg/L. and the results are reported in **Table IV** and **Table V** for *Candida* and *Aspergillus* species respectively. All the tested compounds except compound **3** and compound **4a** of this series showed antifungal activity against one or the other fungal strains used. Compound **6b** and compound **6f** were found to be the most potent compounds of the series. **6b** possessed greater degree of antifungal activity in comparision to standard drugs against *Candida albicans, C.krusei G03*, and *Aspergillus niger*. However, it showed equipotency with standards against *C.parapsilosis 22019*, and *A.flavus*. **6f** showed better activity than the standards against *C.albicans* and it revealed equipotent activity to that of standard against *C.krusei G03*, *C.parapsilosis 22019, A.niger*, and *A.flavus*.

TABLE IV

Pharmacological data of compounds 3, 4a-4g, 5a-5g, and 6a-6g

Compounds	Antifungal activity# [diameter of inhibition zone (mm)]				
	Candida albicans	*Candida albicans ATCC*	*Candida krusei GO3*	*Candida glabrata HO5*	*Candida parapsiolsis 22019*
@ Control	0	0	0	0	0
Fluconazole*	29	25	19	15	20
Griseofulvin*	25	26	18	16	22
3.	-	-	-	-	-
4a.	-	-	-	-	-
4b	-	13	-	15	-
4c	-	11	-	11	-
4d.	-	11	-	13	-
4e.	7	-	-	-	-
4f	-	14	-	14	-
4g	-	11	-	11	-
5a.	13	16	-	-	-
5b.	16	17	13	-	-
5c	11	14	11	-	-
5d.	12	13	-	11	-
5e.	10	-	-	-	-
5f	15	14	13	-	-
5g	11	13	10	-	-
6a.	16	18	11	-	-
6b.	32	22	28	-	22
6c	23	18	21	-	18
6d.	21	17	-	-	17
6e.	15	16	-	-	17
6f	31	22	19	-	19
6g	22	20	15	-	-

 # Concentration was 100 mg/L.
@ 10% DMSO is methanol.
– : No inhibition zone.
** Standard drugs used for comparison.*

TABLE V

Pharmacological data of compounds 3, 4a-4g, 5a-5g, and 6a-6g.

Compounds	Antifungal activity# [Inhibition in percentage]		
	Aspergillus fumigatus	*Aspergillus niger*	*Aspergillus flavus*
@ Control	0	0	0
Fluconazole*	-	90	84
Griseofuloin*	80	88	82
4.	-	-	-
5a.	-	-	-
5b.	-	37	44
5c.	-	34	37
5d.	-	25	-

5e.	-	26	-
5f	-	38	54
5g	-	30	-
6a.	-	32	-
6b.	65	69	71
6c	56	57	66
6d.	-	38	54
6e.	-	36	-
6f.	62	65	67
6g.	-	54	-
6a.	-	65	-
6b.	75	93	82
6c.	68	79	69
6d.	-	85	-
6e.	-	72	-
6f.	73	91	83
6g.	66	53	-

 # Concentration was 100 mg/L.
@ 10% DMSO is methanol.
– : No inhibition zone.
Standard drugs used for comparison.

The characteristic feature of compound **3** and compounds **4a-4g** is the presence of aminothiadiazole ring and substituted arylidineimino moiety respectively; while that of compound **5a-5g** and compound **6a-6g** is the presence of azetidinone and thiazolidinone rings respectively.

Compound **3** was devoid of antifungal activity towards all the fungal strains used, which shows that, presence of thiadiazole ring does not play any role in antifungal activity.

Among compounds **4a-4g** with substituted arylidineiminomoiety, except compound **4a** all the other derivatives exhibited the antifungal activity against some or the other fungal strains used. Out of these seven arylidine derivatives **4b** displayed better activity than rest of the compounds i.e **4a,4c, 4d, 4e, 4f,** and **4g**. This increase in the antifungal activity in these compounds as compared to their parent compound i.e compound **3** may be due to the presence of substituted arylidineimino moiety at two position of thiadiazole ring.

Further conversion of compounds **4a-4g** into their corresponding azetidinone derivatives (**5a-5g**) enhances the antifungal activity. Among these seven derivatives, **5b** with o-hydroxyphenyl group seems to be more efficacious than rest.

On the other hand, cyclisation of compounds **4a-4g** into their corresponding thiazolidinone derivatives produced compounds **6a-6g** with much better and broader spectrum activity than other compounds of this series. This marked increase in these compounds can be attributed to presence of thiazolidinone moiety. It is interesting to note that compound **6b** with hydroxy group at ortho position of phenyl ring and compound **6f** with methoxy group at ortho position of phenyl ring were found to be the most potent compounds of the series. When compared with the standard drugs, compound **6b** exhibited greater activity against *C.albicans, C.krusei G03*, and *A.niger* and was equipotent against *C.parapsilosis 22019*. **6f** exhibited higher activity as compared to standard against *C.albicans* and showed equipotency against *C.krusei G03, C.parapsilosis 22019, A.niger,* and *A.flavus*. The other derivatives i.e **6a, 6c, 6d, 6e,** and **6g** also revealed statistically optimal activity though the activity was lower than the standard drugs.

Experiment

Melting points were taken in open capillary tubes and were uncorrected. Analytical data of C,H,N were within ±0.4% of the theoretical values. IR spectra (cm^{-1}) were recorded on Beckman-Acculab 10 spectrophotometer. ^{1}H-NMR spectra were determined in $CDCl_3$/DMSO-d_6 on Brucker 300-FT instrument using TMS as internal reference standard. Mass spectra were determined on a VG-70-S instrument.

Chemistry

A mixture of 2-amino pyridine (0.1 mol), ethyl chloro acetate (0.1 mol) and anhydrous K_2CO_3 (5.0 g) in acetone (80 ml) was refluxed for about 15 hours on a steam bath. The excess solvent was distilled off under reduced pressure and the resulting solid mass was poured into ice cold water and then filtered. The solid, thus separated, was recrystallised to give compound **1**.

M.P. 88ºC; Yield 83%; Recrystallisation Solvent Ethanol Molecular Formula $C_9H_{12}N_2O_2$.

IR (KBr) ν_{Max} (cm^{-1}): 1725 (C=O), 2844 (CH_2), 3265 (NH_2). ^{1}H-NMR ($CDCl_3$+DMSO-d_6) δ (ppm): 1.25 (t, 3H, $COOCH_2CH_3$), 4.20 (q, 2H, $COOCH_2CH_3$), 4.55 (d, 2H, $NHCH_2$), 7.40 (d, 1H, H_a), 7.49 (dd, 1H, H_c), 7.61 (dd, 1H, H_b), 8.35 (d, 1H, H_d), 9.45 (ss, 1H, $NHCH_2$,exchangeable with D_2O). (Calculated/ Found); % C, (60.00/60.42); % H, (6.66/6.06); % N, (15.55/ 15.39).MS: $[M]^+$ m/z 180.

2-Aminopyridineacetylthiosemicarbazide (2)

Compound 1 (0.025 mol) and thiosemicarbazide (0.025 mol) in methanol (60 ml) was refluxed for 12 hours. The solvent was removed under reduced pressure and the viscous mass was poured over ice cold water, filtered and recrystallised to give compound 2.

M.P. 93ºC; Yield 68%; Recrystallisation Solvent Methanol; Molecular Formula $C_8H_{11}N_5OS$. IR (KBr) ν_{Max} (cm^{-1}): 1165 (C=S), 2840 (CH_2), 3275 (NH_2). ^{1}H-NMR ($CDCl_3$+DMSO-d_6) δ (ppm): 4.52 (d, 2H, $NHCH_2$), 7.38 (d, 1H, H_a), 7.52 (dd, 1H, H_c), 7.60 (dd, 1H, H_b), 8.33 (d, 1H, H_d), 8.75 (m, 4H, $NHNHCSNH_2$), 9.65 (ss,1H, $NHCH_2$). (Calculated/ Found); % C, (42.66/42.79); % H, (4.88/4.65); % N, (31.11/31.24). MS: $[M]^+$ m/z 225.

2-Aminomethylene-(2´-amino-1´,3´,4´-thiadiazol-5´-yl)pyridine (3)

A mixture of thiosemicarbazide and conc. H_2SO_4 (25 ml) was kept overnight at room temperature. The reaction mixture was then poured into ice cold water and neutralized with liquid ammonia and then filtered. The product thus obtained was recrystallised to furnish compound 3.

M.P. 112ºC; Yield 65%; Recrystallisation Solvent Methanol; Molecular Formula $C_8H_9N_5S$.

IR (KBr) ν_{Max} (cm^{-1}): 685 (C-S-C), 1280 (N-N), 1445 (C-N), 1515 (aromatic C····C), 1585 (C=N), 3310 (NH_2), 3330 (NH). ^{1}H-NMR ($CDCl_3$+DMSO-d_6) δ (ppm): 4.48 (d, 2H,$NHCH_2$), 5.85 (bs, 2H, NH_2 exchangeable with D_2O),), 7.39 (d, 1H, H_a), 7.51 (dd, 1H, H_c), 7.65 (dd, 1H, H_b), 8.38 (d, 1H, H_d), 9.63 (ss, 1H, $NHCH_2$). (Calculated/ Found); % C, (46.37/46.25); % H, (4.34/4.32); % N, (33.82/33.68). MS: $[M]^+$ m/z 207.

2-Aminomethylene-(2´-substitutedarylideneimino-1´,3´,4´-thiadiazol-5´-yl) pyridines (4)

A solution of compound 3 (0.012 mol) in methanol was refluxed with aryl aldehyde (0.012 mol) in the presence of few drops of 2% NaOH solution for 10-12 hours. Meanwhile, the completion of reaction was monitored by TLC. The excess of the solvent was removed through distillation. The solid, thus separated, was poured into crushed ice and filtered. The product hence obtained was recrystallised to give comound 4.

2-Aminomethylene-[2´-(p-hydroxy)benzylideneimino-1´,3´,4´-thiadiazol-5´-yl) pyridine (4b)

M.P. 183ºC; Yield 60%; Recrystallisation Solvent Benzene/Water; Molecular Formula $C_{15}H_{13}N_5OS$. IR (KBr) ν_{Max} (cm^{-1}): 675 (C-S-C), 1266 (N-N), 1445 (C-N), 1515 (aromatic C····C), 1585 (C=N), 2845 (CH_2), 3078

(aromatic C-H), 3330 (O-H), 3330 (N-H). ^{1}H-NMR ($CDCl_3$+DMSO-d_6) δ (ppm): 3.10 (d, 2H, NHC<u>H</u>$_2$), 5.79 (bs, 1H, CH_2N<u>H</u>), 7.10-7.45 (m, 4H, Ar-<u>H</u>), 7.49 (d, 1H, H_a), 7.56 (dd, 1H, H_c), 7.68 (dd, 1H, H_b), 8.25 (d, 1H, H_d), 8.35 (ss, 1H, N=C<u>H</u>-Ar), 12.45 (ss, 1H, O<u>H</u>-Ar, exchangeable with D_2O). (Calculated/ Found); % C, (57.87/57.95); % H, (4.18/4.26); % N, (22.51/22.69). MS: $[M]^+$ m/z 311.

Compounds 4a , 4c, 4d, 4e, 4f, and 4g were synthesized from compound 3 by employing an identical method as described for 4b. Physical and analytical data of compounds 4a-4g are given in **Table-I.**

Table I

Physical and analytical data of 2-aminomethylene(2'-substituted arylideneimino-1',3',4'-thiadiazol-4'-yl)pyridines (4a – 4g).

Compd. No.	R	M.P. (˚C)	Yield (%)	Recrystallisation solvent	Molecular Formula	Elemental Analysis (%) Calculated/Found		
						C	H	N
4a.	H	153	62	Methanol	$C_{15}H_{13}N_5S$	61.01/ 61.20	4.41/ 4.35	23.73/ 23.63
4b.	m-OH	185	64	Benzene	$C_{15}H_{13}N_5SO$	57.87/ 57.90	4.18/ 4.16	22.51/ 22.62
4c.	p-OH	212	66	Methanol	$C_{15}H_{13}N_5SO$	57.87/ 57.66	4.18/ 4.12	22.51/ 22.59
4d.	o-$N(CH_3)_2$	202	60	Ethanol	$C_{17}H_{18}N_6S$	60.35/ 60.22	5.32/ 5.40	25.44/ 22.56
4e.	o-OH p-OCH_3	211	62	Methanol	$C_{18}H_{15}N_5SO_2$	59.17/ 59.46	4.16/ 4.28	19.17/ 19.42
4f.	p-OCH_3	210	60	Acetic Acid	$C_{18}H_{15}N_5SO$	61.89/ 61.75	4.29/ 4.15	20.05/ 20.13
4g.	m-OCH_3	200	59	Acetic Acid	$C_{18}H_{15}N_5SO$	61.89/ 61.73	4.29/ 4.21	20.05/ 20.16

2-Aminomethylene-[2´´-(3´´-chloro-2´´-oxo-4´´-substitutedaryl-1´´-azetidinyl)-1´,3´,4´-thiadiazol-5´- yl)pyridines (5)

A stirred solution of compound 4 (0.01 mol) was refluxed in dry DMF (80 ml) containing a small amount of anhydrous $ZnCl_2$ and thioglycolic acid (0.02 mol) for 15-20 hours.The reaction mixture was cooled and poured into ice cold water.The separated solid was filtered, washed and recrystallised to yield compound 5.

2-Aminomethylene-[2´-(3´´-chloro-2´´-oxo-4´´-m-hydroxy benzyl -1´´-azetidinyl) -1´,3´,4´-thiadiazol-5´- yl)pyridine (5b)

M.P. 233ºC; Yield 50%; Recrystallisation solvent Ethanol/water; Molecular Formula $C_{17}H_{14}N_5SO_2Cl$. IR (KBr) ν_{Max} (cm^{-1}): 680 (C-S-C), 1268 (N-N), 1455 (C-N), 1510 (aromatic C<u>····</u>C), 1595 (C=N), 1730 (C=O of β lactam), 2840 (CH_2), 3085 (aromatic C-H), 3326.37 (OH), 3345 (N-H). ^{1}H-NMR ($CDCl_3$+DMSO-d_6) δ (ppm): 3.15 (d, 2H, C<u>H</u>$_2$NH), 4.69 (d, 1H, CH-Cl), 5.72 (bs, 1H, CH_2N<u>H</u>, exchangeable with D_2O), 7.15-7.42 (m,4H, Ar-H), 7.51 (d, 1H, H_a), 7.58 (dd, 1H, H_c), 7.68 (dd, 1H, H_b), 8.22 (d, 1H, H_d), 8.40 (ss, 1H, N-C<u>H</u>-Ar), 12.40 (ss, 1H, O<u>H</u>-Ar). (Calculated/ Found); % C, (52.64/52.43); % H, (3.61/3.52); % N, (18.06/18.25). MS: $[M]^+$ m/z 387.

Compounds 4a, 4c, 4d, 4e, 4f, and 4g were utilized to procure compounds 5a, 5c, 5d, 5e, 5f, and 5g respectively by foregoing methodology for the synthesis of compound 5b. Physical and analytical data of compounds 5a-5g are mentioned in **Table-II.**

Table II

Physical and analytical data of 2-aminomethylene-[2'-(3"-chloro-2"-oxo-4"-substitutedaryl-1"-azetidinyl)-1',3',4'-thiodiazol-5'-yl] pyridines (5a – 5g).

Comp d. No.	R	M.P. (˚C)	Yield (%)	Recrystallisation solvent	Molecular Formula	Elemental Analysis (%) Calculated/Found		
						C	H	N
5a.	H	220	55	Methanol	$C_{17}H_{14}N_5SOCl$	54.91/ 54.78	3.77/ 3.71	18.84/ 18.89
5b.	m-OH	230	52	Ethanol	$C_{17}H_{14}N_5SO_2Cl$	52.64/ 52.53	3.61/ 3.57	18.06/ 18.15
5c.	p-OH	230	55	Benzene	$C_{17}H_{14}N_5SO_2Cl$	52.64/ 52.73	3.61/ 3.58	18.06/ 18.17
5d.	o-$N(CH_3)_2$	234	56	Ethanol	$C_{19}H_{19}N_6SOCl$	55.00/ 55.13	4.58/ 4.39	20.26/ 20.12
5e.	o-OH p-OCH_3	240	58	Benzene	$C_{18}H_{16}N_5SO_3Cl$	51.73/ 51.92	3.83/ 3.72	16.76/ 16.66
5f.	p-OCH_3	201	65	Acetic Acid	$C_{18}H_{16}N_5SO_2Cl$	53.79/ 53.77	3.98/ 3.89	17.43/ 17.53
5g.	m-OCH_3	211	53	Acetone	$C_{18}H_{16}N_5SO_2Cl$	53.79/ 53.67	3.98/ 3.88	17.43/ 17.47

2-Aminomethylene-[2´-(2´´-substitutedaryl-4´´-thiazolidinon-3´´-yl)-1´,3´,4´-thiadiazol-5´-yl)pyridines (6)

To a stirred solution of compound **5** (0.01 mol) and triethyl amine (0.02 mol) in dioxane (5 ml), acetyl chloride (0.02 mol) was added dropwise at 0º-5ºC. The reaction mixture was stirred for about 5-7 hours and the precipitated amino hydrochloride was then filtered off. Further, the filtrate was concentrated under reduced pressure and poured into ice cold water. The product so obtained was recrystallised to furnish compound **6**.

2-Aminomethylene-[2´-(2´´-o-hydroxybenzyl-4´´-thiazolidinon-3´´-yl)-1´,3´,4´-thiadiazol-5´-yl)pyridine (6b)

M.P. 225ºC; Yield 56%; Recrystallisation Solvent.PetroleumEther; Molecular Formula $C_{17}H_{15}N_5O_2S$. IR (KBr) ν_{Max} (cm^{-1}): 683 (C-S-C), 1526 (C····C of aromatic), 1625 (C=N), 1750 (C=O of β-thialactam), 3050 (aromatic C-H), 3335 (OH), 3340 (N-H). ^{1}H-NMR ($CDCl_3$+DMSO-d_6) δ (ppm): 2.75 (s, 2H, CH_2), 3.18 (d, 2H, $\underline{CH_2}NH$), 5.70 (bs, 1H, $CH_2\underline{NH}$, exchangeable with D_2O), 6.12 (s, 1H, C$\underline{H}$-Ar), 7.15-7.45 (m,4H, Ar-H), 7.53 (d, 1H, H_a), 7.59 (dd, 1H, H_c), 7.71 (dd, 1H, H_b), 8.25 (d, 1H, H_d), 12.48 (ss, 1H, O$\underline{H}$-Ar). (Calculated/ Found); % C, (52.98/52.65); % H, (3.89/3.52); % N, (18.18/18.35). MS: $[M]^+$ m/z 385 .

Various other derivatives i.e. 6a, 6c, 6d, 6e, 6f, and 6g were prepared following the same procedure as mentioned for synthesis of 6b. Their physical and analytical data are given in **Table-III.**

Table III

Physical and analytical data of 2-aminomethylene-[2'-(2"-substitutedaryl-4"-thiazolidinon-3"-yl)-1',3',4'-thiadiazol-5'-yl]pyridine (6a – 6g).

Compd No.	R	M.P. (˚C)	Yield (%)	Recrystallisation solvent	Molecular Formula	Elemental Analysis (%) Calculated/Found		
						C	H	N
6a.	H	202	60	Methanol/ Water	$C_{17}H_{15}N_5O_5$	47.11/ 47.38	3.46/ 3.16	16.16/ 16.02
6b.	m-OH	226	56	Ethanol	$C_{17}H_{15}N_5O_2S_2$	52.98/ 52.58	3.89/ 3.36	18.08/ 18.40
6c.	p-OH	241	59	Haxane	$C_{17}H_{15}N_5O_2S$	52.98/ 60.25	3.89/ 4.12	18.18/ 18.00
6d.	o-$N(CH_3)_2$	237	58	Methanol/ water	$C_{19}H_{20}N_6OS$	51.35/ 51.00	4.50/ 4.77	18.91/ 19.19
6e.	o-OH p-OCH_3	244	52	Methanol/ Water	$C_{18}H_{17}N_5O_3S$	48.32/ 48.10	3.80/ 3.60	15.65/ 15.80
6f.	p-OCH_3	246	54	Actic Acid	$C_{18}H_{17}N_5O_2S$	50.11/ 49.95	3.94/ 4.08	16.24/ 16.02
6g.	m-OCH_3	260	58	Methanol	$C_{18}H_{17}N_5O_2S$	50.11/ 50.36	3.94/ 3.69	16.24/ 16.51

Antifungal activity

Compounds were evaluated for their antifungal activity. All these compounds were tested against Aspergillus flavus, A niger and A. fumigatus, C. albicans, ATCC, C glabrata, C.parapsilosis. Candida spp. and C. Krusei at Nicolas Piramal Ltd. Mumbai.

Poisoned food technique (gehlot and vohra, 1998) was performed to evaluate the antifungal property of the test compounds and standard drugs i.e. fluconazole and griseofulvin against *Aspergillus flavus*, *A. niger,* and *A. fumigatus*.

10% solution of DMSO in methanol was prepared. 100 mg of test compound as well as the reference drugs i.e fluconazole and griseofulvin were dissolved in sufficient amount of this solution (5 ml). This solution (5 ml) was added to 995 ml Czapex Dox Agar medium so as to obtain 100 mg /L concentration of the compound in the medium. 5ml of 10% DMSO in methanol solution (without any test compound or the standard drug) added to 995ml Czapex Dox Agar medium served as control. The resultant solutions were thoroughly mixed and approximately 20 ml of the solution was poured into 9cm sterile glass Petridishes and allowed to set. The resulting agar plates were inoculated with 5 mm plugs of fungal mycelia cut from freshly prepared, actively growing cultures. The plates were then incubated at 25 ±˚C in the dark for eight days. The diameter of each colony was measured after eight days of incubation. Three replicates were taken for each test compound and for each organism test culture. The average inhibition due to the given test compound was calculated using the equation:

$$\text{Inhibition \%} = (C-T)\ 100 / C$$

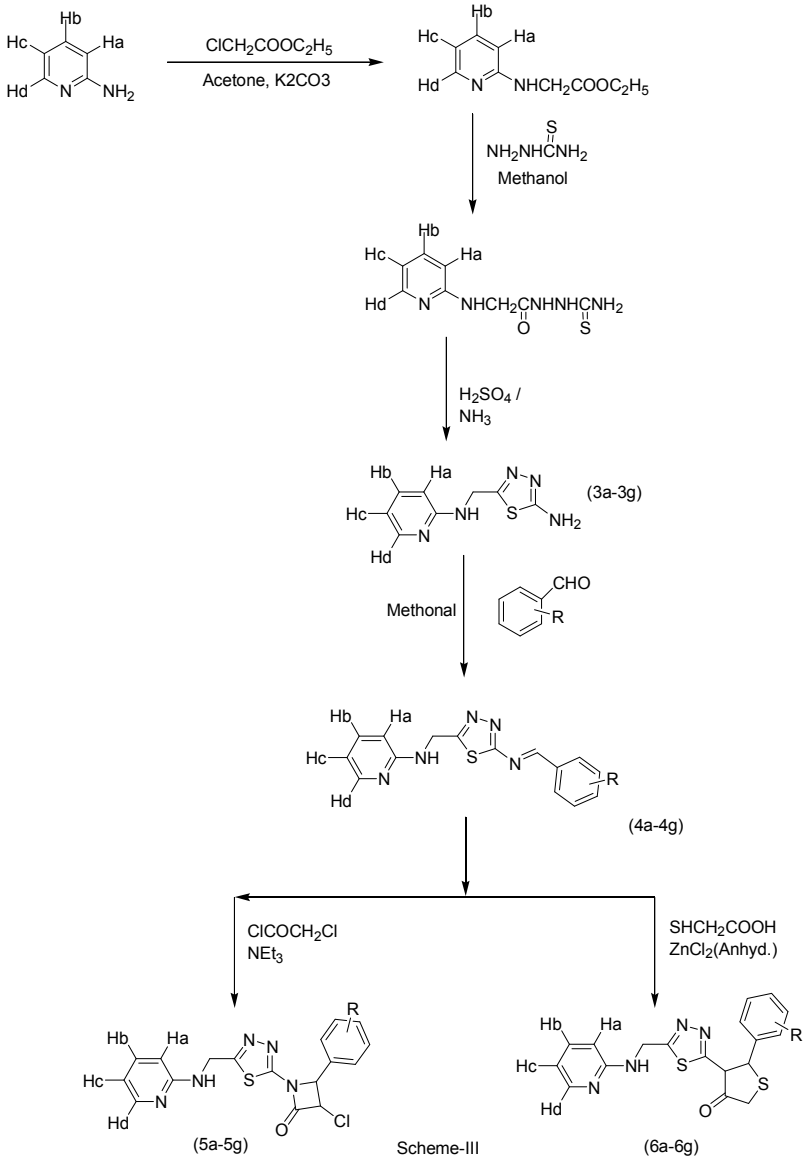
Wherein;

C = Diameter of the fungal colony in mm in the control medium.

T = Diameter of the fungal colony in mm in the test medium containing the given test compound or the reference drug.

Standard agar disc diffusion method (pai and platt, 1995)

All the cultures were maintained on Sabouraud Dextrose Agar medium and incubated at 30˚C. In order to prepare homogenous suspension of these fungi for disc assays, they were grown overnight in Sabouraud broth, centrifuged to collect the pellet and resuspended in sterile phosphate buffered saline. The fungal pellet was homogenized in sterile hand held homogenizer. This suspension was then plated on a Sabouraud Dextrose Agar medium using a bacterial spread to obtain an even growth. Sterile 6 mm whattmann filter paper disc were impregnated with 100mg/ L of various test compounds and standard drugs. These discs were then placed in the centre of quadrant of Sabauroud Dextrose Agar medium plate. These plates have one control disc impregnated with 10% DMSO in methanol. These plates were incubated at 30˚C. Three replicates were used for each test compound as well as for each standard drug used. After 48 hours the plates were removed and the radii of inhibition zone were measured and the average was calculated.



Scheme-III

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